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What Fraction of DNA Double-Strand Breaks Produced by the Direct Effect is accounted for by Radical Pairs?

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Abstract

The purpose of this investigation was to determine what fraction of double strand breaks (dsb), generated by the direct effect of ionizing radiation on DNA, can be accounted for by radical pairs. A radical pair is defined as two radicals trapped within a separation distance of < 3 nm. Q-band EPR was used to measure the yield of radical pairs in calf thymus DNA films X-irradiated at 4 K. The EPR spectrum of DNA showed no evidence of radical pairs. In order to determine the relative sensitivity for radical pair detection via Q-band EPR, we measured the yield of radical pairs, $G(\text{rp-Thy})$. In single crystals of thymine, $G(\text{rp-Thy})$ was $\sim 8 \text{ nmol/J}$, under the same conditions employed for DNA. The value of $G(\text{rp-Thy})$, in conjunction with the measured signal-to-noise, was used to calculate an upper limit for the yield of radical pairs in DNA, $G_{\text{max}}(\text{rp-DNA}) < 0.7 - 1.4 \text{ nmol/J}$. The upper limit, $G_{\text{max}}(\text{rp-DNA})$, was compared with the yield of dsb, $G_{\text{total}}(\text{dsb}) = 10 \text{ nmol/J}$, previously measured in pUC18 DNA films by Purkayastha, S.; Milligan, J. R.; Bernhard, W. A. *Radiat. Res.* **2007**, *168*, 357. We found that $G_{\text{total}}(\text{dsb}) > 2 \times G_{\text{max}}(\text{rp-DNA})$, implying that a significant fraction of dsb were not derived from a pair of trappable radicals. At least one of the two precursors needed to form a dsb was a diamagnetic (molecular) product. The hypothesis is that EPR silent lesions are formed through a molecular pathway. For example, a two-electron oxidation of deoxyribose would result in a deoxyribose carbocation intermediate that ultimately leads to a strand break.

INTRODUCTION

The mechanisms of radiation-induced double strand breaks (dsb) in DNA are of long standing interest. This is partly because, among the many occurring types of complex lesions, dsb are simplest to detect. More importantly, it is because the high toxicity of ionizing radiation is directly related to the high efficiency with which radiation forms complex lesions. A number of dsb mechanisms have been proposed: (i) single radical¹, (ii) soliton², (iii) pair of radical ions^{3,4}, (iv) two radicals born within an ionization cluster⁵, and (v) dissociative electron capture⁶. Of these, evidence supports (iv) or a combination of (iii) and (iv), as the dominant mechanism^{7,8}. Therefore, dsb formation entails two (or more) lesions spanning both strands and separated by no more than $\sim 3 \text{ nm}$ (10 base pairs).

When the opposed lesions are both prompt single strand breaks (ssb), a prompt dsb is formed. If an ssb or damaged base is opposite another damaged base, then excision of the damaged base by a repair enzyme creates a dsb⁹. In the case of the direct effect, the predominant lesions are formed via the following free radical intermediates: a carbon-centered deoxyribose radical due to the loss of a hydrogen from one of the five carbon sites,

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a guanine radical cation, a thymine radical anion, and a cytosine radical anion, respectively^{10,11}. A two-radical dsb mechanism entails the formation of a precursor consisting of two radicals, with one radical on each strand and separated by a distance d (Figure 1a) where d is considered to be ~ 10 base pairs (~ 3 nm). If these two closely spaced radicals exist at the same time, they form what is known in electron paramagnetic resonance (EPR) as a radical pair and they are detected by EPR with a sensitivity comparable to that for detecting isolated radicals. The aim of this work is to determine what fraction of dsb, prompt plus enzymatically induced, can be accounted for by radical pairs.

Radical pairs are characterized by a strong electron-electron coupling, creating a highly anisotropic interaction described by a tensor $\mathbf{D}$. The magnitude of the coupling, D , depends on the angle θ between the applied magnetic field $\mathbf{B}$ and the vector $\mathbf{d}$ connecting the two radicals and also on the distance between the two radicals. Because D is proportional to $1/d^3$, the unpaired electron-electron interaction decreases quickly with distance. When $d > \sim 3$ nm the radicals no longer give rise to radical-pair spectral features and are referred to as monoradicals. The formation of radical pairs and dsb, therefore, share the same spatial requirements. If a two radical mechanism is the main precursor to dsb, then radical pairs should exist as intermediates. By irradiating DNA at low temperatures, $= 77$ K, radical intermediates are trapped and evidence of radical pairs are obtainable through EPR.

One can test for radical pair formation by looking for an EPR signal at half field ($g \approx 4.006$), where the $\Delta m_s = 2$ transition occurs (simultaneous flip of both electrons) or by looking at $g \approx 2.003$, where the $\Delta m_s = 1$ transitions occur for both the paired radicals and the monoradicals. The former has the advantage that any signal at 4.006 is almost assuredly due to radical pairs and the latter has the advantage that the radical pair signal is more than an order of magnitude stronger. Symons and coworkers looked for, and found no evidence of, radical pairs in DNA at 77 K^{12,13}. But for this null result to take on meaning, two quantities are needed. One is the yield of total dsb, $G_{\text{total}}(\text{dsb})$, and the other is the minimum yield of radical pairs needed for detection by EPR. If the latter is greater than the former, then it is possible that all dsb are formed via the two radical mechanism. Here we fill in the missing information by measuring the detection threshold for radical pairs and comparing it with recently measured dsb yields.

After γ -irradiation of pUC18 films at 279–285 K, Yokoya et al¹⁴ measured a prompt dsb yield of $G(\text{dsb}) = 4 \pm 1$ nmol/J for DNA hydrated to a level, referred to as Γ , of 14.5 mol $\text{H}_2\text{O}/\text{mol}$ nucleotide. After X-irradiation of pUC18 films at 4 K, Purkayastha et al¹⁵ found $G(\text{dsb}) = 4.0 \pm 0.6$ nmol/J for DNA at $\Gamma = 15$. For this comparison, we converted the yield reported by Yokoya et al from one based on a target mass of DNA alone to one based on DNA plus the mass of its solvation shell. Using enzymes to reveal base damage, it was also possible to measure dsb that involved reduced pyrimidines and oxidized purines, which in the Purkayastha work added 1.5 ± 1.3 and 2.1 ± 1.3 nmol/J, respectively, to the dsb yield. Summing over all types of dsb, the total yield for the work of Purkayastha et al, was $G_{\text{total}}(\text{dsb}) = 7.6 \pm 1.9$ nmol/J. For the work of Yokoya et al, $G_{\text{total}}(\text{dsb}) = 18 \pm 5$ nmol/J. Based on these yields, if dsb are initiated by a two radical intermediate, the yield of radical pairs in DNA, $G(\text{rp-DNA})$, will be about twice $G_{\text{total}}(\text{dsb})$. The factor of two comes from the probability of radical pair formation on one strand, as in Figure 1b, being equal to formation on opposite strands. Thus, only half of the total radical pairs would give rise to dsb. Based on the findings of $G_{\text{total}}(\text{dsb})$ being in the 8–18 nmol/J range, we should expect $G(\text{rp-DNA})$ in the 16–36 nmol/J range. Our aim here is to determine the threshold for radical pair detection, $G_{\text{max}}(\text{rp-DNA})$, and by comparison with $G_{\text{total}}(\text{dsb})$ determine what fraction of the dsb yield can be accounted for by the radical pair yield.

To our knowledge, the yield of radical pairs produced by ionizing radiation has not been reported for any material. But there are materials where the radical pair signal was reported to be relatively strong. One such material is crystalline thymine, as can be seen from Figure 3 of the work by Boon et al.¹⁶. Irradiation of single crystals of thymine at 77 K has been shown to produce two types of radicals, one due to the net loss of a H of the methyl group, Thy(Me-H)[•], and the other due to the net gain of a H at C6, Thy(C6+H)[•]¹⁷. Furthermore, there are at least two types of radical pairs in these crystals, both types consisting of Thy(Me-H)[•] radicals¹⁸. The more prominent type has the interconnecting vector **d** lying in the *bc* crystallographic plane and a separation *d* (measured from C6 of one radical to C6 of the other) of 0.76 nm. The other type has **d** lying in the *a***b* plane and *d* of 0.65 nm¹⁸. The maximum coupling, *D*_{max}, for the two types is 28 mT and 36 mT, respectively. Because of the large coupling *D*, the $\Delta m_s = 1$ signal of the radical pair is easily detected even in the presence of the signal due to the monoradicals trapped in the same sample. The radical pair signal and monoradical signal are both centered at $g = 2.0025$ but, because the monoradical signal has a spectral width of < 14.5 mT, any signal in the outer wings of the spectrum must be due to radical pairs. These properties make crystalline thymine advantageous for quantifying radical pair production in DNA.

The aim of the work reported here is to use radical pair formation in crystalline thymine as a standard against which the following question can be answered. Is the yield of free radical pairs in DNA sufficient to account for the yield of dsb?

MATERIALS AND METHODS

Sample preparation

Calf thymus DNA (ctDNA), used as purchased from United State Biologicals, was dissolved in nuclease free water to 7–9 $\mu\text{g}/\mu\text{L}$ and the concentration was determined by absorbance at 260 nm. Aliquots of the ctDNA solution were pipetted into open-ended silylated suprasil quartz tubes and then dried in sealed chambers either against P_2O_5 or a saturated solution of NaOH. Under these conditions, it is assumed that DNA contains ~ 2.5 mol water/mol nucleotide. Once dried, the films were weighed and subsequently taken to a higher level of hydration by allowing them to equilibrate for at least 3 weeks in a chamber having a relative humidity of 84%, controlled by a saturated solution of KBr. By this protocol, the hydration level of DNA is assumed to be ~ 15 mol water/nucleotide¹⁹. The film weights were measured periodically with a Cahn C-30 Microbalance to an accuracy of ± 1 μg . The level of film hydration, Γ (moles of water per mol nucleotide), was calculated from the difference in the weight of the pre- and post- equilibrated films. The films were then transferred to Charles Supper Co. quartz tubes, chosen for the low free radical background produced upon irradiation, for free radical trapping studies. Based on our previous work {Purkayastha, 2007 #7381; Sharma, 2008 #7392, DNA damage produced by the procedure itself is negligible.

Single crystals of anhydrous thymine were kindly provided by William Nelson, Georgia State University. For single crystal measurements, the crystals were fit snugly into a 1.0 mm Charles Supper tube. Polycrystalline samples were prepared by grinding single crystals and pressing the material into pellets ~ 1 mm in diameter and ~ 3 mm long and were also placed in Charles Supper tubes.

EPR spectroscopy

The concentration of trapped radicals at 4 K was measured using a Q-band (35 GHz) Varian E-12 EPR spectrometer operating at a nominal microwave output of 20 mW attenuated by 50 db and using an oriented ruby crystal as an internal standard mounted inside the EPR cavity {Mercer, 1987 #4160}. The films were X-irradiated at 4 K and then raised into the

EPR cavity and allowed to equilibrate for several minutes before spectra were recorded at 4 K. All EPR spectra were taken as first derivatives. The data were collected at $g = 2.003$ using 1000 points per EPR scan, typically spanning a field width of 40 mT. Approximately 30 mT of the scan was devoted to the EPR spectrum of the sample, the remainder being devoted to the spectrum of the ruby standard. Following a simple ramp baseline correction, the spectrum was numerically integrated to obtain the absorption spectrum. This was baseline corrected and integrated again, yielding the area under the absorption curve, which is the signal intensity. The baseline correction and integration procedure was also carried out on the ruby portion of the EPR spectrum to determine the intensity of the ruby standard. Free radical concentrations were then determined by comparing the intensity of the EPR signal at 4 K to that of the ruby standard at 4 K for each of the samples at each dose point.

The signal to noise (S/N) levels for crystalline thymine and ctDNA were measured in each case by dividing the peak-peak value for the signal by the peak-peak value for the background scan. Peak-peak was measured by taking the difference between the maximum up and the maximum down excursion. These S/N values were used to determine the limits for radical pair detection in the samples.

Chemical yields

The X-ray source was a Varian/Eimac OEG-76H tungsten-target tube operated at 70 keV and 20 mA, and the X-ray beam was filtered by 40 μm thick aluminium foil. Dosimetry was previously described²⁰. The dose rate inside the Charles Supper capillary was 45 kGy/hr. The dose regime extended from 0 to 175 kGy for thymine crystals and from 10 to 50 kGy for ctDNA.

For the ctDNA, the free radical concentrations were based on the presumed target mass consisting of just DNA, 15 waters per deoxynucleotide and one sodium counterion bound per deoxynucleotide. For the crystalline thymine samples, the chemical yields were based on the entire sample mass. In the case of DNA, the fraction of the target mass attributable to just DNA was obtained by measuring the absorbance at 260 nm, by dissolving the ctDNA film (after the experiment) in a known amount of nuclease free water.

RESULTS

Yield of radical pairs in crystalline thymine

Radical pairs were readily detected in single crystals of thymine irradiated at 4 K and observed at 4 K by Q-band EPR. Figure 2 shows that portion on the radical pair signal that extends beyond the strong central signal. The predominate radical pair has an electron-electron coupling D of 18 mT at this orientation. The central signal is primarily due to monoradicals but must also contain some signal due to radical pairs. Following on previous studies performed at 77 K with X-band EPR^{16,18}, the magnetic field, B_0 , was set parallel to the crystallographic c -axis so as to maximize the separation of the radical pair signal from the monoradical signal. Because the radical pair signal-to-noise (S/N) was far better for the $3m_s = 1$ signal ($g = 2.003$) than the $3m_s = 2$ signal at half-field ($g = 4.006$), our measurements focused on the $3m_s = 1$ signal. The concentration of all trapped radicals, isolated and paired, was measured over a dose range 0 to 175 kGy. This dose response, shown in Figure 3, is linear over the entire range. From the slope of the fitted line, the chemical yield of all trapped radicals in single crystals of Thy, $G(\text{fr-Thy})$, was found to be $15.9 \pm 0.4 \text{ nmol/J}$.

In order to obtain the yield of radical pairs in Thy, $G(\text{rp-Thy})$, we first determined what fraction of the radicals that were trapped in pairs were of the type with a separation distance of 0.76 nm and characterized by $D_{\parallel} = 28 \text{ mT}$. This was done by double integrating the wing lines and comparing that intensity with that of entire spectrum of Thymine. We found the

wing lines account for ~ 6% of the total trapped radical population. As pointed out in the Introduction, there is at least one other type of radical pair that has been observed at 77 K, but by inference it appears to be at significantly lower concentration¹⁶. Under our conditions, we see no clear evidence of another radical pair type, which should be apparent at crystal orientations in the *ab* crystal plane where *D* reaches 36 mT¹⁸. In order to err on the conservative side, we assumed that we missed half of the total radical pair population when partitioning the *c*-axis spectrum. Thereby, we conclude that the total radical pair population accounts for no more than 12% of the all trapped radicals in thymine. This gave a maximum yield for all types of radical pairs in thymine single crystals of $G(\text{rp-Thy}) < 1.8 \pm 0.04 \text{ nmol/J}$.

Because our DNA films are amorphous, not single crystals, it was important to determine the *S/N* for radical pair detection in a sample with all radicals at random orientations. Therefore, we took measurements on polycrystalline thymine and, as can be seen in the top of Figure 4, the radical pair signal is readily detected. The total free radical yield was $G(\text{fr-Thy}) = 17.7 \pm 1.5 \text{ nmol/J}$ and, using the value of 12% maximum radical pairs determined above, the radical pair yield was calculated to be $G(\text{rp-Thy}) = 2 \pm 0.2 \text{ nmol/J}$. Further, the *S/N* of the radical pair signal increased linearly with dose, as expected. The values of *S/N* for thymine radical pairs are given in Table 1 for 50 kGy and 10 kGy; these are the doses chosen to search for radical pairs in DNA.

Yield of radical pairs in ctDNA films

The EPR spectrum of ctDNA at 50 kGy is shown in the middle of Figure 4. As seen in the overlay at the bottom of Figure 4, there is no evidence of the wing line features that are expected for radical pairs. The importance of this null result resides in its use to calculate the maximum possible yield of radical pairs in DNA, $G_{\text{max}}(\text{rp-DNA})$.

From our work on thymine, *S/N* = 1 is sufficient for detection of radical pairs. In DNA, we assumed detection would be at a *S/N* between 1 and 2. Using noise figures measured for DNA and thymine, and using $G(\text{rp-Thy}) \leq 2 \pm 0.2 \text{ nmol/J}$, we calculated the values for $G_{\text{max}}(\text{rp-DNA})$ at *S/N* equal to 1 and 2. The threshold for detection of radical pairs in DNA, given in Table 1, are in the 0.1 – 0.2 nmol/J range at a dose of 50 kGy and in the 0.4 – 0.9 nmol/J range at 10 kGy. The significance of these $G_{\text{max}}(\text{rp-DNA})$ values lies in the comparison with dsb yields produced in DNA under the same conditions.

DISCUSSION

The yield threshold, $G_{\text{max}}(\text{rp-DNA})$, for detection of radical pairs in ctDNA, X-irradiated and observed at 4 K, was found to be 0.1 – 0.9 nmol/J (Table 1). For pUC18, X-irradiated under the same conditions and hydrated to the same degree ($\Gamma = 15 \text{ mol H}_2\text{O/mol}$ nucleotide), the yield of dsb, $G_{\text{total}}(\text{dsb})$, due to both deoxyribose and base damage was $7.6 \pm 1.9 \text{ nmol/J}^{15}$. In order to compare $G_{\text{max}}(\text{rp-DNA})$ with $G_{\text{total}}(\text{dsb})$, two factors must be taken into account: i) not all paired radicals yielded dsb and ii) not all paired radicals were detected.

One half of the radical pairs are expected to reside on the same strand and would, therefore, not lead to dsb (as illustrated in Figure 1). Therefore, if all dsb were derived from trappable radical pairs, the yield of radical pairs should be twice the dsb yield, i.e., $15 \pm 4 \text{ nmol/J} \approx 2 \times 7.6 \pm 1.9 \text{ nmol/J}$, as given in Table 1. Not all of these radical pairs would be detected. For optimal detection of radical pairs, D_{max} should be > 10 mT. In crystalline thymine, the predominant pair gives $D_{\text{max}} = 28 \text{ mT}$ with the intra-pair distance of $d = 0.76 \text{ nm}^{18}$. Since D_{max} is proportional to $1/d^3$, our detection method would miss radical pairs with $d > 1.07 \text{ nm}$. The distance corresponding to 10 bp of B-form DNA is $9 \times 0.34 \text{ nm} = 3.06 \text{ nm}$. We

should, therefore, be able to detect $\sim 1/3$ (1.07/3.06) of the radical pairs formed within a 10 bp segment of DNA, assuming that the probability of radical pair trapping is independent of d . In other words, this detection criteria allows for the possibility that $\sim 2/3$ of the radical pairs relevant to dsb formation are missed. With this factor taken into account, we arrive at the yield of detected radical pairs expected if all dsb are produced via a trappable radical pair, which is 5 ± 1 nmol/J as given in Table 1. The observed $G_{\text{max}}(\text{rp-DNA})$ of < 1 nmol/J then argues that no more than 20% of the dsb can be accounted for by trappable radical pairs.

Conversely, we conclude that a large fraction ($>80\%$) of dsb are formed via two unstable intermediates on opposite strands, one or both of which must be a non-radical (i.e., diamagnetic damage). This finding is consistent with recent discoveries that the yield of ssb^{15,21} and free base release^{20,22,23} exceeds the yield of free radicals trapped by the deoxyribose-phosphate backbone. The fraction of ssb or free base release that cannot be accounted for by trappable radicals varied from 30–70%. If 50% of ssb in DNA stem from a trappable radical, then simple combinatorial statistic would predict that 25% of the dsb yield stems from a trappable radical pair. The shortfall in radical-pair precursors to dsb found here, therefore, is consistent with the shortfall in monoradical precursors to ssb reported previously. In the above referenced works, it was hypothesized that the non-radical damage is a carbocation produced by double oxidation.

The lack of radical pairs in DNA should not be surprising based on a large body information on radical trapping in crystals of DNA constituents and related compounds^{24,25}. Low LET radiation deposits about half its energy via ionization and about half via excitation in materials such as DNA and H_2O ²⁶. Radical pairs in DNA can potentially be formed via these two different pathways i.e., one initiated by ionization and the other by excitation. Each ionization produces a radical cation (a hole) and a radical anion (an electron gain center). Since a large fraction of ionizations occur within clusters, there is the possibility of trapping radical pairs, i.e., two radicals separated by $d < \sim 3$ nm. But in order to trap a radical pair, each radical must be relatively immobile and thereby not lost to recombination.

Materials that favor radical pair formation are materials that favor homolytic bond cleavage over ionization²⁷. Bond cleavage is one fate of excitations, produced either by primary energy depositions or by recombination reactions. In materials that are poor traps for ion radicals, increased recombination increases the yield of excited states and thereby increases the probability of homolytic bond cleavage. Also, because relaxation of the excited state quenches bond dissociation, materials that promote long lived excited states are more likely to undergo homolytic bond cleavage. Crystalline thymine is such a material while DNA is not.

In DNA, pathways initiated by ionization dominate. Of the radicals initially formed, about half are lost to re-combination; they are not trapped even at 4 K²⁸. In round numbers, the yield of trapped radicals is ~ 600 nmol/J, and the loss of radicals due to combination reactions is ~ 600 nmol/J. If a small fraction, $\sim 1/10$, of these combination reactions are not back reactions whereby the electron returns to a hole, but forward reactions generating double oxidation or double reduction sites, then the yield of diamagnetic damage will be significant even at 4 K and even in very short times at 300 K.

A dsb mechanism that entails either two radicals, or one radical and one diamagnetic damage, or two diamagnetic damages is consistent with the experimental results obtained by Prise et al⁸ and Milligan et al⁷. Both of these experiments rely on competition kinetics with thiols. If the reactivity of the diamagnetic damage, e.g., carbocations, mimics that of the radicals, then their conclusions still hold true but in broader terms. The strand breaking

precursor pairs can be any combination of two unstable intermediates: one a radical and the other a non-radical that may well be formed by two one-electron oxidations at the same site.

CONCLUSION

The radical pair yield in crystalline thymine at 4 K was found to be $G(\text{rp-Thy}) = 2 \pm 0.2$ nmol/J. Based on the radical pair yield in thymine, the S/N figure for Q-band EPR spectra in thymine, and the lack of a detectable EPR signal for radical pairs in DNA, the maximum yield of radical pairs in DNA, X-irradiated and observed at 4 K, was found to be 0.1 – 0.9 nmol/J. From this threshold for detection of radical pairs trapped in DNA, we conclude that < 20 % of double strand breaks are formed in DNA via radical pairs. At least one of the two damages is not a radical. It must be, therefore, a molecular product. The hypothesis is that EPR silent lesions are formed through a molecular pathway; for example, a two electron oxidation of deoxyribose would result in a deoxyribose carbocation intermediate that ultimately leads to a strand break.

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Figure 1.

(a) Represents two radicals (•) present on opposite strands (—) of DNA, at a distance $d \leq 3$ nm, giving rise to dsb. **(b)** Represents two radicals present on the same strand of DNA, at a distance $d \leq 3$ nm, giving rise to ssb.

Figure 2.

The first-derivative, Q-band EPR spectra of a thymine single crystal recorded at 4 K after X-irradiation to a dose of 175 at 4 K. The crystal is aligned with the magnetic field, B_0 , approximately parallel to the c-axis. At this orientation, the predominate radical pair has an electron-electron coupling D of 18 mT. The modulation amplitude was 0.4 mT and the microwave power attenuation was 50 dB. Ruby was used as an internal standard to determine the free radical concentration; its signal lies about 40 mT up field of the thymine signal .

Figure 3.

Dose-response curve for total radicals trapped in thymine, X-irradiated and measured by EPR at 4 K. The data has been fitted to a straight line by least squares and the total radical yield, 15.9 ± 0.4 nmol/J, was determined from the slope of this line. About 1/4 of the total radical yield is due to radical pairs, ~ 4 nmol/J.

Figure 4.

The first-derivative, Q-band EPR spectra recorded at 4 K after X-irradiation at 4 K. At the top is polycrystalline thymine after a dose of 90 kGy. In the middle is ctDNA after a dose of 10 kGy. At the bottom, the spectrum of ctDNA is compared with that of polycrystalline thymine recorded after the same dose, 10 kGy.

Table 1

The maximum radical pair yield in ctDNA films, calculated from the measured radical pair yield in crystalline thymine, is less than half of the radical pair yield predicted if all dsb are derived from radical pairs.

Sample	Dose (kGy)	Measured Radical Pair Peak- Peak S/N	Measured Radical Pair Yield (nmol/J)
Thymine (polycrystalline)	50	13 ± 3	$\leq 2 \pm 0.2$
	10	3 ± 0.6	$\leq 2 \pm 0.2$
		Assumed S/N Threshold for Radical Pair Detection	Maximum Radical Pair Yield
ctDNA (film)	50	1	0.1 ± 0.03
		2	0.19 ± 0.06
	10	1	0.43 ± 0.13
		2	0.85 ± 0.27
pUC18 DNA (film)	Conditions		Measured dsb yield^a
	deoxyribose damage only		4.0 ± 0.6
	deoxyribose + base damage		7.6 ± 1.9
			Predicted Radical Pair Yield^b
	Probability of radical pair spanning opposite strands = probability of radical pair on same strand		15 ± 4
	Radical pair separations must be < 3 bp to be detected. This is ~1/3 of the radicals within the presumed 10 bp distance that result in dsb.		5 ± 1

^aValues from Purkayastha et al ¹⁵

^b Assumed that radical pairs on the same strand do NOT give rise to dsb.